RECEIVED CENTRAL FAX CENTER JAN 1-1 2007

Amendments to the Specification.

Please amend the paragraph bridging p.15-16 as follows:

Expression of Listeriolysin O and Target Proteins. To facilitate expression of mature cytoplasmic LLO in E. coli, the hly gene encoding LLO lacking its N-terminal signal sequence (22) was closed into the plasmid vector pACYC184 to generate pDP3615 as described in Materials and Methods. Transcription of the truncated hly gene in pDP3615 is under the control of the constitutive tet gene promoter. Proteins to be delivered to the cytosol of macrophages were expressed from co-resident plasmids in E. coli. We chose chicken ovalbumin (OVA) as one of the representative proteins to deliver to the cytosol of macrophages. OVA is not toxic to E. coli and can be readily expressed to high levels (32). A plasmid encoding truncated (32kD) OVA was transformed into E. coli along with pDP3615. In order to determine if a large protein with a measurable enzymatic activity could be delivered to the cytosol of macrophages, we expressed βgalactosidase ( $\beta$ -gal) along with LLO in E. coli. A plasmid containing the gene encoding  $\beta$ -gal, was transformed into E. coli along with plasmid pDP3615. Expression of both OVA and β-gal in these strains is under the control of IPTG-inducible phage T7 RNA polymerase. We next analyzed the hemolytic activity and protein expression profiles of these strains. Following IPTG induction, OVA and β-gal were expressed to approximately 20% of the total E. coli cellular protein as determined by SDS-PAGE. To verify expression of active LLO protein within E. coli, hemolytic activity contained in the soluble fraction of E. coli extracts was determined as described above. All of the strains expressing LLO contained approximately 500-600 hemolytic units of activity in the soluble extracts. No measurable hemolytic activity was found in the culture medium in which the E. coli were grown. These data indicate that functional LLO protein was contained within the E. coli cells and not secreted to the extracellular environment.